Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

David L Sedlak

Final Selection Panel Review

Proposal Title

#0111: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Funding:

Fund in part
Amount: **$1,167,149**

The final Selection Panel concurred with its initial findings on this proposal and recommended funding the proposal at the reduced amount recommended as a result of those deliberations. Should the California Bay−Delta Authority accept the Selection Panel's recommendation and approve the funding of this proposal, the applicant will be allowed to negotiate which tasks and associated costs will be reduced as part of the contracting process.

Public Comments

No public comments were received for this proposal.

Initial Selection Panel Review

Proposal Title

#0111: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Funding:

Fund in part
Amount: **$1,167,149**

Initial Selection Panel (Primary) Review

Topic Areas

- Life Cycle Models And Population Biology Of Key Species
- Environmental Influences On Key Species And Ecosystems
- Relative Stresses On Key Fish Species
- Salmonid−related Projects

Please describe the relevance and strategic importance of this proposal in the context of this PSP. How does the proposal address the topic areas identified above? What are the broader CALFED Goals this proposal may meet that are not accounted for in these specific topic areas?

Winter−run salmon sex ratios have varied substantially though time and recent work has found relatively high incedences of sex reversal in male chinook salmon. This proposal will identify known sex−disruptive compounds, focussing on agriculrtural discharges, and correlate them with indicators of hormone disruption in salmon. Information on sex reversal is critical to population models of salmon and information on the nature and distribution of hormone disrupting compounds promises a powerful management tool that we do not currently have any handle on.

The budgets of proposals submitted in response to this PSP are larger, on average, than those submitted to CALFED in previous years. The Science Program is committed to getting as

much science per dollar as is reasonably possible. With this commitment in mind, can the proposed budget be streamlined? If so, please recommend and clearly justify a new budget total in the space provided.

Several reviewers comented on the relatively high cost of the proposal but none really offered suggestions on ways to cut costs. Some suggestions were made that low-cost additions to some aspects would add value and that some low cost parts were not as valuable, so it is probably worthwhile to cosult with the proponents before awarding the grant and try to come up with a maximally useful proposal. Alternatively, perhaps a reduced cost proposal could be developed.

Evaluation Summary And Rating.

Provide a brief explanation of your summary rating and any additional comments you feel are pertinent.

Xenoestrogens are a hot and happening field of study and have been raised as a significant management issue on the Columbia and elsewhere; but in teh Central Valley we have only information that concern is warranted. This proposal takes us from that state of alarm into a state of response and therefore is very attractive. It is a little disturbing that some reviewers comment on teh appropritaeness of one part of the study and others suggest that that is the part that is most questionable; the reviewer that refers to Coho salmon throughout his review of this project dealing with Chinook salmon does not fill me with confidence about the quality of his review. Nevertheless, the general agreement is that the proposal is very worthwhile and likely to achieve its goals.

Selection Panel (Discussion) Review

fund this amount: $**1,167,149**
note:
fund in part

The Panel felt that this project was relevant and timely. The management implications of this research are clear. The

project team appears to be well–qualified. The Panel appreciated the plan to merge correlative field studies with an experimental lab–based approach. This proposal is very management–oriented although it is expected that further identification of the putative feminizing agents will be required. The Panel felt that this proposal represented an important first step in addressing a potentially significant problem.

The Panel felt that this proposal and proposal #318 were complimentary and that the two project teams, working collaboratively, would likely produce more valuable products than if they were funded independently. Specifically, the value of proposal #318 (a valuable project in its own right) is enhanced by the environmental context provided by the proposal considered here. The Panel believes both studies should be funded as a single project with an integrated workplan. The Panel believes that the budget for the proposal considered here (#111) can be reduced by 10% form what proponents' requested.

Panel Ranking: Fund with modification

Collaboration Panel Review

Proposal Title

#0111: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Final Panel Rating
above average

Collaboration Panel (Primary) Review

Collaboration:

Will the results of the collaborative effort be greater than the sum of its parts? Is it clear why the subprojects are part of a larger collaborative proposal rather than several independent smaller ones?

This $1.3 million proposal joins UC Berkeley, Riverside, Applied Marine Sciences, Southern Nevada Water Audthority to quantify xenoestrogens in Delta tribuataries through chemistry, in vitro bioassays, and in vivo assays for feminization in salmon. It then identifies the source of the xenoestrogenic activity, and exposes salmon to natural waters with different levels of xenoestrogens to see if feminization can be induced. The tools developed with the salmonids are then used for other fish species in the watershed.

Interdependence And Integration:

Does the proposal have an example that clearly articulates the conceptual model of each subproject and how they link together as a whole? Are the boundaries of the study plans focused and cohesive, yet well delineated? Is there a plan for potential differences in the stages of subproject completion times? Are there clear plans for analyses and interpretations which seek to identify and quantify relationships among the data collected in various subprojects rather than separate analyses for each subproject?

The design of the tasks moves back and forth between

chemistry, toxicology, and field validation that provides for internal integration.

Project Management:

Is it clear who will be performing management tasks and administration of the project? Are there resources set aside for project management and time given for investigators to collaborate? Is there a process for making decisions during the course of the project? Are there acknowledgments of potential barriers to collaboration and explanations of how team members will overcome barriers particular to their institutions?

Management is explicitly discussed—though not budgeted as a separate task. A specific individual is named, is budgeted for ensuring project details get covered, and has experience with two of the team members. There is a good discussion of possible problems and how to resolve them.

Team Composition:

Does the lead principal investigator have successful management history and experience leading collaborative teams? Is it clear that all key personnel are committed to making significant contributions to the project? Do team members have complementary skills?

Evidence was presented that the PI has managed large projects. The team has a proven history of collaboration. All of the PIs have significant budgeted participation.

Communication Of Results:

Is there a clear plan for comprehensive and cohesive reporting of project progress to the CALFED community?

Besides the usual mix of papers and talks, the team proposes a creative use of cross-department seminars among other related CalFed projects as a communications tool. The group also makes use of an external advisory panel that includes a manager from the Central Valley Board, but there's no one included from the pesticide user community. The team also proposes a project

website

Additional Comments:

Collaboration Panel (Discussion) Review

Primary reviewer judges that the collaboration greatly enhanced the value of this project. Project management is specifically addressed, but not budgeted as a task, however. He felt that the management statement is overly simplistic but overall it is a good creative collaborative project, with some creative communication of results.

Secondary reviewer agreed with the primary reviewer, and felt that the proposal is a good mix of new organizations and collaborations.

Technical Synthesis Panel Review

Proposal Title

#0111: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Final Panel Rating
above average

Technical Synthesis Panel (Primary) Review

TSP Primary Reviewer's Evaluation Summary And Rating:

This proposal builds on a growing body of information in the scientific literature that relates anthropogenic contaminants in water to feminization of fish species in receiving streams. The principal hypothesis of the proposal is that observed feminization of Chinook salmon (other studies cited in the proposal) in the CA bay-delta system has a similar origin, and that agricultural drainage, urban and commercial water discharges may be responsible. The approach prposed builds on similar studies in other parts of the world, especially Europe, using a water fractionation and bioassay procedure to identify potential estrogencic contaminants at selected sites within the Delta. The methods proposed appear to be appropriate although some details of the procedures to be used are missing, which frustrated efforts of external reviewers to fully understand the approach. The results of the work will likely be interesting and informative regarding the potential source of the decline of salmon in this system, but the reviewers focus on the complexity of the monitoring and bioassay studies and the difficulty that may arise in establishing a definitive cause and effect relationship between levels of contaminants and observed feminization in Chinook salmon in the BD system. Further studies are probably going to be needed to translate this work into management

actions. One of the external reviewers notes that what is missing is an assay/measures which would be important for modeling/remediation. The team is well qualified and the budget, while large, is needed for such an ambitious sampling and analysis program.

Additional Comments:

This proposal builds on a growing body of information in the scientific literature that relates anthropogenic contaminants in water to feminization of fish species in receiving streams. The principal hypothesis of the proposal is that observed feminization of Chinook salmon (other studies cited in the proposal) in the CA bay−delta system has a similar origin, and that agricultural drainage, urban and commercial water discharges may be responsible. The approach prposed builds on similar studies in other parts of the world, especially Europe, using a water fractionation and bioassay procedure to identify potential estrogencic contaminants at selected sites within the Delta. The methods proposed appear to be appropriate although some details of the procedures to be used are missing, which frustrated efforts of external reviewers to fully understand the approach. The results of the work will likely be interesting and informative regarding the potential source of the decline of salmon in this system, but the reviewers focus on the complexity of the monitoring and bioassay studies and the difficulty that may arise in establishing a definitive cause and effect relationship between levels of contaminants and observed feminization in Chinook salmon in the BD system. Further studies are probably going to be needed to translate this work into management actions. One of the external reviewers notes that what is missing is an assay/measures which would be important for modeling/remediation. The team is well qualified and the budget, while large, is needed for such an ambitious sampling and analysis program.

Technical Synthesis Panel (Discussion) Review

TSP Observations, Findings And Recommendations:

The proposal addresses an important topic that has been widely–studied in Europe and less–studied in the US or, in particular, the Bay–Delta Ecosystem. The applicants cite a substantial rate of feminization among salmon in this ecosystem, indicating that this may be an important area for study. The research team is well–qualified to conduct this work; that is, in part, because these techniques and questions are not new. Had the applicants proposed a study that targeted more cutting–edge issues (e.g. specific feminization agents, their sources and means for control), the panel would have rated the proposal "superior". Admittedly, there is potential for this study to produce new scientific discoveries and, if so, it will certainly address an emergent management issue in this ecosystem. External reviewers identified several methodological issues; however, these were viewed mostly as differences in opinion regarding the best possible approach to the research questions – not serious concerns over the adequacy of the methodologies proposed. Overall, the four external reviews rated this proposal very highly with few critical comments.

Technical Review #1

proposal title: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**Are the goals, objectives and hypotheses clearly stated and internally consistent? The goals are clearly stated, but the body of the proposal flips between salmon and using surrogate species in field testing. This should have been more clearly stated in the initial goals. In addition, one of the goals stated initially is to make recommendations for cost−effective measures to reduce EDC inputs into the watershed. However, this goal is not addressed later in the proposal.** **Is the idea timely and important? Yes, this is very timely and important.**
Rating	**very good**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**Is the study justified relative to existing knowledge? Yes, there is quite a bit of preliminary evidence showing that there is a feminization problem in the Chinook salmon, and there are numerous suspect chemicals.**

	Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Yes, the conceptual model is clearly laid-out, including sampling scheme and potential for xenobiotics to impact developing salmon. **Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified? There is enough preliminary data to justify a full-scale project.**
Rating	**excellent**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	**Is the approach well designed and appropriate for meeting the objectives of the project? The approach is fairly well designed. The YES assay is questionable as a screen for EDCs since the yeast tend to pump certain classes of xenobiotics back out of their systems, and therefore the YES assay is limited in it's use. Using an E-Screen or other in vitro system that does not have the YES assay drawbacks would have been better. Since this is a crucial screen to identify potentially estrogenic chemicals to then further assay in vivo, this is a large drawback to this proposal.** **The other approaches (in vivo assays, chemical analysis) seem reasonable.** **Is the approach feasible? Yes.** **Are results likely to add to the base of knowledge? Yes.**

	Is the project likely to generate novel information, methodology, or approaches? There are several new methods that could be developed from this proposal, and the information generated will certainly be novel. **Will the information ultimately be useful to decision makers? This information will be extremely useful to decision makers, especially if cost−effective prevention measures can be proposed to regulators. Identifying which chemicals are the primary culprits in various portions of the watershed will be extremely useful.**
Rating	**very good**

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	**Is the approach fully documented and technically feasible? Yes, the approach is definitely feasible, with experts in their respective fields participating in this project. It may be quite tricky to tease out which chemicals exactly are causing the feminization, especially if there is more−than−additive effects, but the authors are well−equipped to handle this scenario.** **What is the likelihood of success? Very high.** **Is the scale of the project consistent with the objectives and within the grasp of authors? The scale is consistent, but it is unclear whether the authors have the engineering background to propose low−cost measures to curb EDC inputs (final objective).**
Rating	**very good**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Yes, the monitoring is appropriately designed, yet leaves room for flexibility if it is needed once the initial round of data is collected.** **Are there plans to interpret monitoring data or otherwise develop information? Yes, there are plans to share the data with relevant peers.**
Rating	**excellent**

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	**Are products of value likely from the project? Yes, especially identification of EDCs in the watershed, and the ability to differentiate between different chemicals that may be the source of feminization in different parts of the watershed will be incredibly useful. In addition, novel combinations of chemicals may be found, leading to a greater understanding of EDC impacts on fish populations.** **Are contributions to larger data management systems relevant and considered? Yes, there are plans to share the data and make it publicly available.** **Are interpretive (or interpretable) outcomes likely from the project? Yes. This is a hypothesis−driven proposal with concurrent laboratory in vitro and in vivo assays to verify effects seen in the field.**

Rating	**excellent**

Additional Comments

Comments	**Overall, this proposal is well-thought out and would be useful in explaining the large percentage of feminized salmon found in this watershed. The identification of specific chemicals will be quite difficult, but using a sample fractionation and screening test (YES assay) followed by a bioindicator test will help differentiate which chemical(s) are important. One of the objectives is to come up with cost-effective measures to reduce specific EDC inputs. The authors do not seem to have the expertise in environmental engineering to do this task. However, there is good interaction with both Advisory Board members and other CALFED institutions, so that ultimately such a recommendation can be developed by decision makers (not necessarily the authors of this proposal).**

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**What is the track record of authors in terms of past performance? The authors are experts in their fields and have had numerous prior interactions. In fact, this proven track record of collaborations in the past is a strength of this proposal.** **Is the project team qualified to efficiently and effectively implement the proposed project? Yes.** **Do they have available the infrastructure and other aspects of support necessary to accomplish the project? Yes, although money is requested to purchase a GC/MS/MS for the high number of samples that will be**

	processed for this proposal.
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**Is the budget reasonable and adequate for the work proposed? The budget seems a little “padded”, but then things are likely more expensive in California than in other parts of the country.**
Rating	**very good**

Overall

Provide a brief explanation of your summary rating.

Comments	**Overall, this proposal is timely and well-thought out. There is enough flexibility to change sampling locations and surrogate species as is necessary if the data prove to be different from that anticipated. Some of the proposed assays are not the best choice (e.g. the YES assay), however there is back-up with the in vivo assays. It could also be quite difficult to delineate which chemicals are acting as EDCs, however this is also one of the important products from this proposal. This information is very important in restoring the health of the salmon population in this watershed.**
Rating	**very good**

Technical Review #2

proposal title: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**There is growing concern regarding the impacts of chemical contaminants on growth, reproduction and survival of aquatic species. The goal for this research is to investigate the causes of feminization of Chinook salmon in the Sacramento and San Joaquin river system: waters that discharge in the San Francisco−San Joaquin delta. Specific objectives to accomplish this goal are clearly stated and include field sampling, chemical analysis, and laboratory bioassays.**
Rating	**excellent**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**A conceptual model is presented that is sufficiently robust to demonstrate the need for this research. The model describes both vulnerable salmonid life stages and their spatial and temporal occurrence in the Sacramento and San Joaquin river system. In addition, there is documented evidence of the presence of xenoestrogens at environmentally relevant levels in**

	these waters. Based on this model and previous findings and full scale study is justified.
Rating	excellent

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	The approach is in two phases; Phase I will consist of field sampling and analysis to identify the presence of selected xenoestrogens. Sampling will target both industrial and agricultural sources. Sampling will also use knowledge of fish biology as outlined in the conceptual model to target sampling in locations where early life-stage salmon exist. The proposed sampling scheme is robust, verging on over ambitious. In addition there appears to be a disproportionate emphasis on agricultural sources as apposed to urban (sewage treatment outfalls, storm drains) and industrial. Within the goal of identifying causative agents, Phase II proposes to refine the sampling scheme and the in vivo bioassay based on findings in Phase I. This two-phase approach uses state-of-the-art analytical techniques, a proven screening assay (the YES assay) and a novel bioassay-directed fractionation fish assay to identify the causal agents.
Rating	excellent

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	More detail on field sampling methods, analytical methods, and bioassay methods would be useful in

	evaluating feasibility. However sufficient information was provided to determine that proposed field sampling and chemical analysis are feasible. The proposed use of the YES assay as a screening tool also is feasible. However, it was difficult to determine the feasibility of the bioassay directed fractionation based on previous work as none of the cited references that describe the fractionation process appear in the bibliography.
Rating	**very good**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**Monitoring is a focus of the proposed research.**
Rating	**not applicable**

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	**Identification of xenoestrogens responsible for feminization in Coho salmon is the main product of this research. A multifaceted approach is used combining rigorous analytical methods and a tiered bioassay scheme. Results of this research should also guide future studies to better understand the relationship between chemical contamination and feminization of other sensitive fish species.**
Rating	**excellent**

Additional Comments

Comments	**For in vivo bioassays a dose response study should be considered; exposing fish to extracts equal to and greater than the concentration found in natural waters.** **The choriogenin assay does not fit well main trust of the proposed research and should be eliminated. Similarly, expansion of in vivo bioassays in Phase II to other fish species (Delta smelt and tule perch) may detract from developing a robust bioassay for salmon.**

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**The principle contributors (Sedlak, Schlenk, Spies) have strong track records. Snyder and Kolodziej are promising scientists possessing track records with an impressive trajectory.**
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**The budget seems appropriate for a project of this duration and scope. However, subcontracting personnel costs for sampling and analysis do stand out. Of particular concern are the personnel costs for sampling, as the subcontractor is a private consultant and there is no opportunity for cost share. Three thousand dollars for office supplies and report preparation seems excessive.**
Rating	**very good**

Overall

Provide a brief explanation of your summary rating.

Comments	**This is an ambitious proposal with high merit and the potential to make a substantial contribution to the body of knowledge regarding the impacts of chemical contaminants on growth, reproduction and survival of aquatic species; specifically in the area of the role of xenoestrogens in the feminization of Coho salmon.**
Rating	**excellent**

Technical Review #3

proposal title: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**An ambitious project is outlined in this proposal that outlines a large scale sampling program over a relatively large geograpical drainage basin. Water samples will be chemically analyzed for known and potential unidentified xenoestrogens and correlated to in vitro and in vivo bioassys of steroid hormone disruption. These goals are well presented with clear objectives outlined in the two primary task phases of the proposed research. Some validation studies and tests will be needed early in the first phase of the project, but these should lead to more refined technical methodologies being applied to the large scale sample analyses. The background section(1.1) presents the hypothesis that xenoestrogens are not only associated with wastewater discharges but may be deriving fron agricultural pesticides and associated dispersing agents as well as from farm animal wastes themselves. The introduction presents a sufficient data set on fish sex reversal in the region as well as updated data on the nature and sources of at least a portion of xenoestrogen loading. Unknown estrogenic agents may also be present as well as the realization that these**

	compounds represents a complex mixture of variable nature and input source. It is likely that not all chemical causes can be identified but the sampling, chemical analyses and bioassays may identify the predominant sources though sampling may have to be adjusted in phase 2. The principle species of concern is native salmon but incluces other important regional fish species also. In conjunction with other CALFED sponsored projects related to chemical contaminants such as agrichemicals and other sources there is a serious need for a full comprehensive environmental assessment of chremical stressors in this large drainage basin which is being severely impacted by humasn activities. If this sex reversal phenomenon affects fertility and reproductive success of the "lynchpin" species then there is a serious problem indeed. What is missing though is an assay/measures of this aforementioned question which would be important for modeling/remediation. The proposers could add this component to the study.
Rating	**very good**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**Obviously in a complex mixture world of environmental contaminants a lot mor research is indeed needed. The authors propose an extensive sampling campaign followed by fractional chemical extractions coupled to steroidogenic bioassays in vitro and in vivo. The experimental approach is designed to evaluate such**

	real world "mixtures", though a better understanding of additive and synergistic interactions between xenoestrogens in such environmentally relevant concentrations still lags in the scientific literature. The conceptual model presented in part 1.3 is well presented and shown in Figure 3. The model focuses on the early life stages of the target species which is indeed likely the most sensitive developmental stages to xenoestrogenic compounds. The model also presents likely sources of contaminant input in the drainage system subregions and addresses the dynamics as well. The selection of sampling sites is well stated and based upon known contaminant information and likely routes of exposure. A full scale project is justified and a significant number of sites selected, though rangefinding and fine tuning will have to be performed in a timely manner early in phase 1.
Rating	**very good**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	**The scientific approach appears well designed to meet the stated objectives. Having Applied Marine Sciences in charge of all field site sample collections should greatly facilitate accomplishing the objectives. Site selection appears well grounded and carefully placed relative to potential sources of estrogenic substances of various type and complexity. Technologically, the chemical analysis of water samples for xenoestrogens is well stated and established and when coupled to the bioassays studies should work in**

identifying chemical fractions displaying estrogenic activity. The bioassays will start by using a YES assay which is a scientifically sound method for the initial activity screens. The in vivo assays are also well established methods. In the area of aquatic toxicology, knowledge pertaining to xenoestrogens still lags behind the database available for other forms of biological effects of toxins. There still exists some degree of controversy related to the biological relevance of some previous bioindicators of estrogeic effects. Vitellogenin and choriogenin assays are clearly better than assays to measure induction of the estrogen receptor as they are more indicative of an "effect" bioindicator. From this perspective, the proposers are on more solid ground. An issue not fully addressed is whether the feminization of male fish affects their fertility and reproductive capacity. This is a most interesting question of big relevance. Adding this component to the design is feasable and might give a big boost to predictive power. The incorporation of some additional treatment groups using environmentally relative concentrations of mixtures of known and putative xenoestorgens would add a "real world" component to the study and possibly address potential additive/synergstic actions in model development. As such, the project should generate considerable field data on contaminant concentrations and distributions relative to source inputs. Potential new estrogenic contaminants may be identified. There should also be some additional methodological refinements to the chemical analysis and bioassay methods, though well established, should be improved. Additional

	new research components, previously suggested, could be easily couched into this study and remain within budget. If there is indeed a serious threat to salmon/fish reproductive capacities at the population level then decision makers need this information for remedial purposes especially when coupled to other sponserd projects pertaining to environmental contaminants in this region
Rating	**very good**

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	**As outlined in the proposal, the approach is very well documented and supported by data shown in the background information section. The methodologies for sampling, chemical analysis and exposure/bioassays are well cited in the references. I am quite familiar with the bioassays and chemical analysis methods and am quite confident in the skills of the scientists involved. I am especially familiar with Dr. Schlenk's research and confident in his labs abilities in this area of molecular bioindicators. The chemical analysis to be performed in the Sedlak lab will utilize an interesting but very efficient solid phase extraction and fractionating methods. Their described protocols for the chemical analysis using GC/MS/MS is the best approach easpecially for the large numbers of replicate samples to be analysed. Their are a lot of dispersed field sites but I am convinced in the efficacy of the field sampling team. One of the strongesty components of the whole propsal is the QA/QC documentation and the setup for communication among the researchers. I would have liked to see a timeline figure showing the timeframe for completing all tasks as well as the timings for various meetings**

	and communications including visits with the blue ribbon advisory board. This is well stated though in textual form. I think there will be significant sucesses but I would also suggest that if funded, the proposers add some additional components into the exposure/bioassay design to guage the reproductive question and mixtures effects.
Rating	**very good**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**This is the strongest part of the proposal. There is a good QA;/QC plan in place and I am confident in the experience of this team in "Chain of Custody" matters regarding samples etc. Because of the wide range of field sites and sampling involves significant liters of water with replicates. Sediment samples seem not really needed as the fish species of interest are primarily of the "Water Column" type of existence. The schedule of tea and lab meetings is good and periodic group meeting will be conducted. A yearly "retreat" or two would be very productive alongside an active advisory panel of outside experts. This apprears fully in place, though a calendar schedule should have been included. A common web site(s) will also be in place. Positive/negative controls and spiked samples are sufficient in all analyses and the exposure/bioassay experiments are well designed and should easily be monitored.**
Rating	**excellent**

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the

project?

Comments	**The benefits from the successful completion of this project should not nercessarity be of a specific product nature as the methodologies are well developed. The major benefits are the database of xenoestrogenic contaminants in geographical and temporal distribution as well as a better predictive model of biologicasl/ecological effects though some addition questions such as reproductive effects on salmon/fish species is critical and not fully addressed in the study. This could be addressed by adding some additional components to the bioassay part is feasable from a cost standpoint. The most important deliverables will be identification of sources and xenoestrogens at strategic field sites relative to potential source. Remedial actions could then be implemented. This information should also be integrated with other databases under study in regards to additional biological effects produced by diverse contaminant classes.**
Rating	**very good**

Additional Comments

Comments	**None**

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	**THis is an excellent team who I am very familiar with. The collaborators are scientifically very sound andfully equipped to begin ASAP. The past performance of the authors on related projects is excellent. I would have suggested Dr. Schlenk and Sedlak from the go as they are the best in this area in your regionand especially in regards to the chemical classes and**

	bioassays under consideration. They have tramed up with Applied Marine Sciences to conduct the sampling component as they are equiped and experirnced for this type of work which cannot be performed "on the cheap". The specialized HPLC/MS/MS analyses to be performed by Dr. Snyder at the Southern Nevada Water Authority is to be conducted in a fully equiped laboratory. The labs have the major analytical equipment for all components of the study and mostly would require expendables. The P.I.'s are well esatablished in the scientific literature as evidenced by their extensive publication records. The overall lab capabilities are excellent.
Rating	**excellent**

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**The budget is reasonable and provides a complete accounting of costs for all the participants. If possible, some additional effects bioassays could be incorporated by Schlenk and associates to address the mixture and reproductive questions discussed in previous sections. The chemical analyses of the many samples is reasonable and cost effective using the GC/MS/MS approach with solid phase extraction cartridges. Frequently, chemical analytes are expensive and yet this component cannot be avoided in this study. The experienced Sedlak and Snyder labs are well experienced and equiped for this project**
Rating	**excellent**

Overall

Provide a brief explanation of your summary rating.

Comments	

	Overall, this is a very good prposal based upon the qualifications of a very experienced and equiped team of collaborators. The sampling and chemical analysis components of the study are very straight forward in scope and deliverables. The in vitro YES assay will work very well in the phase 1 sample water screens. Site selection and GIS use is also a considerable strength of this proposal and is built on a good database in regards to xenoestrogens though this is not as strong as for other types of adverse effects resulting from chemical exposures. The exposure/in vivo assays are very good but could be expanded to incorporate some additional bioindicators to assess potential reproductive impairment which can be extroplated to regional salmon populations and fits in well with the CALFED programs that focus on Salmon species as well as other vitally important speciues in this large drainage system. I funded fully, I believe some additional bioindicators can be couched into this part of the study. Both phases of the project and most deliverables can be accomplishe within the timeframe of the project
Rating	**very good**

Technical Review #4

proposal title: Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	**The goals and objectives are clearly stated and consistent through the grant. The research is timely and important, as the identification of xenoestrogens that may reduce the fecundity of of salmon would provide a basis for proper environmental management.**
Rating	**excellent**

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full−scale implementation project justified?

Comments	**The justification is well described and the conceptual model clearly states the basis for the proposed work.**
Rating	**excellent**

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	

Overall, the approach is well designed and appropriate for the meeting the objectives. However the reviewer has a few reservations. The authors state in the Background section that 38% of the male Chinook salmon have been feminized based on measuring sex reversal. The authors do not plan to measure sex reversal, but instead vitellogenin and choriogenin levels. These are reasonable measures of exposure to environmental estrogens, but are these biomarkers associated with increased sex reversal? There is no mention of research indicating an association or correlation of the biomarkers to sex reversal. Secondly, since sex reversal is an obvious physiological perturbation that would affect fecundity, it may be in the best interest of the authors to measure sex reversal in addition to vitellogenin. The reviewer recognizes the difficulty in monitoring sex reversal, as vitellogenin and choriogenin induction may be transient due to transient release of xenoestrogens and sex reversal may be a slow, continuous process that is not easy to correlate with xenoestrogen release.

It is the reviewers opinion that the sites should be pre−selected based on the GIS program described. See below for comments on monitoring.

The YES assay methodology could be better described. The estrogen receptor used is not mentioned. I guess that it uses a human or rodent estrogen receptor, and not a fish receptor, but this is not mentioned. This is an important point since a fish (trout, salmon) estrogen receptor may demonstrate different affinities for some xenoestrogens than that of a mammal. The estrogen receptor is reasonably conserved. However, if it is a mammalian receptor, is there data demonstrating that the affinities of most xenoestrogens are similar between fish and mammals?

The TIE analysis is well described and appears adequate to determine the chemicals that induce feminization. The in vivo bioassay is also well

	described, except for the in vivo assay to be performed with sediments (see below).
Rating	**very good**

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	**For the most part the approach appears technically feasible. However, the authors do not describe how they might conduct the sediment in vivo bioassays and therefore it is difficult to discern the feasibility of the sediment approaches.** **The likelihood of success is very good and within the grasp of the authors. They provide adequate description of the work for the most part, and the authors are excellent scientists as demonstrated in their CVs.**
Rating	**very good**

Monitoring

If applicable, is monitoring appropriately designed (pre−post comparisons; treatment−control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	**The monitoring needs to be better described within the grant. Exactly how many times do the researchers propose to visit the sites, what months, what years, what sites, etc. A table should be provided to show how many samples will be collected from each of the sites and when. This would help the reviewer determine the number of samples that must be analyzed.** **The authors also need to describe in detail how sites would be selected. The authors state "we will use a**

	GIS-based mapping tool that plots annual use patterns of specific types of pesticides at a one mile spatial resolution". The reviewer would like a better description of the how the model works. Better yet, the model would already have run and the sites selected.
Rating	good

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	
Rating	not applicable

Additional Comments

Comments

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	The authors of the grant are preeminenet environmental chemists and toxicologists. The project team has also worked together in the past, and therefore demonstrate a clear ability to collaborate. Furthermore, more information in the YES assay and its estrogen receptor would be appreciated.
Rating	excellent

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	**The budget is reasonable for the work proposed.**
Rating	**excellent**

Overall

Provide a brief explanation of your summary rating.

Comments	**The research is important research and could identify toxicants that are initiating feminization in fish. The goals are well justified and this type of research is necessary in making proper management decisions. However, collection sites have not been chosen and the method of choosing them is not well described.** **Furthermore, there is no data demonstrating a correlation or association between vitellogenin, choriogenin and sex reversal. The biomarkers suggested are excellent at demonstrating xenoestrogen exposure, but there ability to determine xenoestrogen effects are still putative. Since sex reversal is the "real" problem, but probably difficult to correlate to xenoestrogen exposure, it may be in the best interest of the authors to measure xenoestrogen loads (as described), vitellogenin, choriogenin (as described), and sex reversal. This may provide a important scientic discovery.** **The authors have decided to take on an important, but difficult project. The reviewer would like to stress that its impact and identification of xenoestrogens and xenoestrogen sources is important.**
Rating	**very good**